Iranian Journal of Pharmaceutical Sciences
2022: 18 (3): 253-264
www.ijps.ir

Original Article

Possible Rapid Therapeutics for SARS Coronavirus 2 RNA-dependent RNA polymerase: A Systematic Review

Vahid Asghariazar[a,c], Kamyar Mazloum Jalali[b], Ali Aghebati-Maleki[c], Somayeh Biparva Haghighi[d], Negin.Balar[b], Hadi Peeri-Dogaheh[e,f], Chiman Karami[e*]

[a] Cancer Immunology and Immunotherapy Research Center, Ardabil University of Medical Sciences, Ardabil, Iran. [b] Department of Microbiology and Medical Virology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran. [c] Deputy of Research & Technology, Ardabil University of Medical Sciences, Ardabil, Iran. [d] School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. [e] Department of Microbiology, Parasitology, and Immunology, Ardabil University of Medical Sciences, Ardabil, Iran. [f] Zoonoses Research Center, Ardabil University of Medical Sciences, Ardabil, Iran.

Abstract

Research confirms that viral polymerases play a key role in viral genome replication and transcription. Hence, they are essential for the multiplication and survival of viral particles. Our review is limited to original papers in the English language from 2019 to 2020 using various databases, including PubMed, Scopus, Google Scholar, and Science direct**.** Out of 159 papers, 27 drugs may effectively prevent *RNA-dependent RNA polymerase* (*RdRP),* of COVID-19, agreed in order of drug discovery years and paper publication, respectively. In this paper, we realized that the structure comparison and sequence alignment suggest that the mode of substrate *RdRP* is highly conserved in diverse RNA viruses, providing a foundation for designing broad-spectrum antiviral drugs based on nucleotide analogs.

Keywords: Conservative, RNA viruses, RNA polymerase.

1. Introduction

Viral infections are common over the world and can vary from minor infections to global outbreaks. Viral infections can lead to massive and deadly diseases. One of the most severe pandemics is SARS-CO2. According to classification, coronaviruses belong to subfamily coronavirinae, family coronaviridae, order Nidovirales, currently known as respiratory viruses [1]. So far, seven human coronaviruses have been documented, namely Severe Acute Respiratory Syndrome coronavirus (SARS-CoV), Middle East Respiratory Syndrome coronavirus (MERS-

Corresponding Author: Dr. Chiman Karami, Department of Microbiology, Parasitology, and Immunology, Ardabil University of Medical Sciences, Ardabil, Iran,
E-mail: chkarami.chiman@gmail.com and c.karami@arums.ac.ir
Cite this article as: Asghariazar V, Mazloum Jalali K, Aghebati-Maleki A, Biparva Haghighi S, Balar N, Peeri-Dogaheh H, et al., Possible Rapid Therapeutics for SARS Coronavirus 2 RNA-dependent RNA polymerase: A Systematic Review, Iran. J. Pharm. Sci., 2022, 18 (2): 253-264.

CoV), HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1. Four viruses (HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1) are responsible for about one-third of common colds ranging from mild to life-threatening infections [2]. Viral genome replication and transcription are catalyzed by viral polymerases. Thus, it is very extremely to characterize the survival and multiplication of viral particles. About two-third of the CoV genome from the 5′-end two large, 5′-terminal ORFs1a and 1ab, which are cleaved by viral proteases to produce the RNA-dependent RNA polymerase (RdRp) are also known as Nsp12 and helicase (Hel). RdRp is the main enzyme of multiportion replicas transcriptase complex essential for transcription and replication of CoVs.

One of the effective strategies to inhibit RdRp and Hel's production is to target the viral proteases. This strategy is integrated in FDA-approved drugs. The success results from the possibility of accommodating interactions between the host and viruses during their mutations and continuously presenting the host with new challenges [3]. The main factor that confirm the role of RdRps in viral evolution and function is the high rate of error during copying (10^4) as no error-correcting processes occur. High mutation rates in viral progeny population permit some variants to be selected under specific circumstances by host defense mechanisms and other environmental influences [4]. Also, the development of strand switching by RdRps through reproduction permits for recombinations that enable readjustment of genes or achievement of new ones from other viruses or hosts [5]. RdRps are considered multi-domain (α and β) database of proteins, which are affiliated to the Structural Classification of Proteins (SCOP) class 2.7.7.48.RdRps. RdRps provide complete structural and evolutionary associations with all proteins whose structures are identified [6]. The beginning of synthesis may happen at the 3'-end of the template in a primer-dependent or independent mode and continues in the 5′→3′ direction. Usually, the core RdRp domain's average length commonly consists of 500 amino acids and is folded encompassing three subdomains, palm, thumb, viz, and fingers resembling a right-handed cup [6].

The way SARS-CoV-2 has emerged and affected peoples' lives necessities an urgent need for antiviral strategies and diagnostic targeting. Conserved function and mechanism of RNA regions have already been shown to play critical roles in the life cycles of coronaviruses. All the viral polymerases depict identical structural topographies and catalytic actions. SARS-CoV RdRp includes several conserved motif C (Leu758_Ser759_Asp760_Asp761) at the active site which is absent in SARS- CO2. They also have important alterations that reflect varied virus replication approaches. However, viral polymerases have also emerged as potential antiviral targets for drug design to treat viral infections.

2. Materials and Methods

The project was approved by the ethical code IR.ARUMS.REC.1399.352, according to the ethical principles and the national norm and standards for conducting medical research in

Iran. For this review, 159 articles from PubMed, Embase and Google Scholar, and Scopus Library have been investigated. The articles were related to diagnoses, vaccines, and therapeutic endeavors. Concerning the inclusion and exclusion criteria and eliminating duplications, 27 studies were finalized for a full review (**Figure 1**). A Google search for 2019-nCoV treatment (as of 6 February 2020; Fig.1) generated five webpage links from the research center and international bodies with official information and guidelines (WHO, Europe CDC, US CDC, US FDA), three webpage links on diagnostic protocols and scientific annotations, and five webpage links on market news and press statements. Google search for

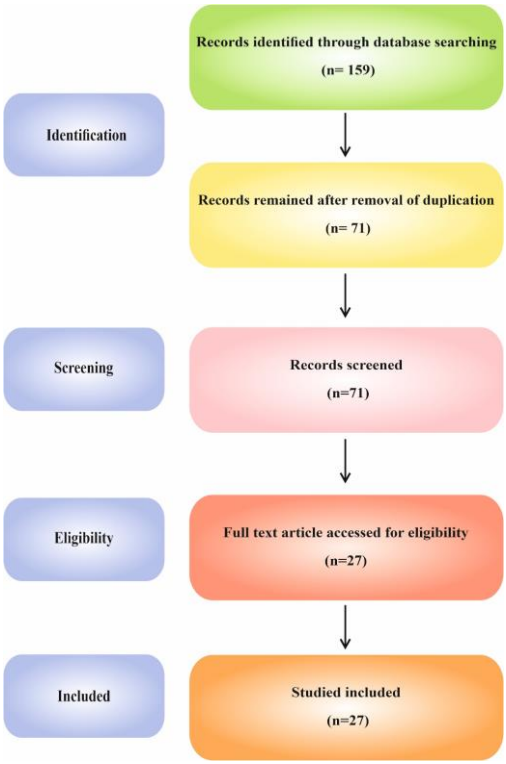

Figure 1: Summary of the standard four-step protocol for the research papers selection.

2019-nCoV treatment produced 27 related articles.

3. Results and Discussion

Out of 159 papers, 27 papers were finally selected for the aim of this investigation (**Table 1**). Accordingly, 27 drugs were found useful in impeding RdRp of COVID-19, presented in order of drug discovery date and paper publication. RdRp plays an essential role in the RNA virus life cycle and has no host cell homolog. This avenue for antiviral drug development reduces the risk that a protein in human cells will be affected. Commonly, viral RdRps are regarded as low-fidelity enzymes, mainly due to the lack of proofreading roles. So far, an extensive range of chain terminators or mutagenic nucleoside analog inhibitors have been explored [7] and it is documented that nucleoside analogs in adenine or guanine derivatives block viral RNA synthesis for a broad spectrum of RNA

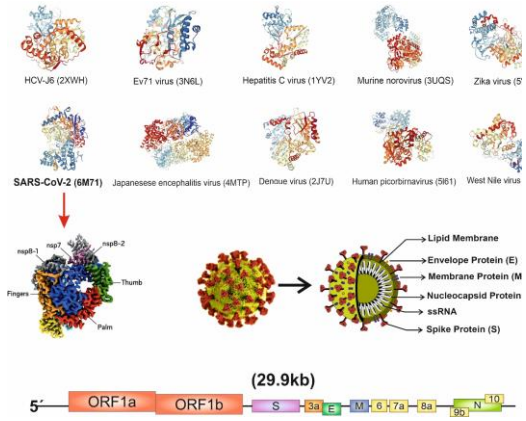

Figure 2: A similar RdRp 3D structure is found in viruses that belong to different classes.

viruses, including human coronaviruses (**Figure 2**) [8].

3.1 Anti-COVID drugs

3.1.1 Favipiravir

A broad-spectrum *antiviral agent* is a guanine analog antiviral drug targeting RdRp of

RNA viruses. Through a *compound library* screen against Influenza RNA-dependent RNA polymerase (RdRp). Favipiravir is now approved in Japan and China, and the its therapeutic program is within *evaluation* process in the United States in the Phase 3 of clinical trial for the treatment of uncomplicated influenza [9]. Favipiravir is a prodrug that undergoes phosphoribosylation and phosphorylation to be in an active form, favipiravir-ribofuranosyl-5-triphosphate (F-RTP), in human cells [10]. F-RTP is recognized as a purine nucleotide by RdRP, but it blocks RdRP enzyme activity and, thus, blocks viral RNA transcription [9].

Table 1. RdRP Inhibitors for Treatments of Viral Infections and probably effective at SARS-CoV-2

Antiviral agents	Year	Reported Mechanism of Action	Approved Clinical Use	Authors	Ref.
Ribavirin	1985	Inhibits viral RNA synthesis and mRNA capping	RSV, HCV, and viral, Hemorrhagic fever, Randomized trial for COVID-19	Graci et al.	(40)
Sofosbuvir	2013	Binds to Mg2+ ions in RdRp of HCV and inhibits HCV replication	HCV genotype 2 or 3	De Clercq Li	(41)
	2014	Binds to the catalytic domain of RdRp and prevents the inclusion of nucleotides for viral RNA replication	Influenza viruses, Randomized trial for COVID-19	Furuta et al.	(9)
	2014	Binds to the palm-1 (P1) site of the influenza virus RdRp to stop virus replication	HCV genotype 1	Eltahla et al.	(42)
	2014	Binds the NS5B (HCV RdRp) thumb pocket-1 allosteric site to inhibit RNA replication	Phase 2 for HCV and HIV/HCV co-infection in combination with a unaprevir and daclatasvir	Lemm et al.	(43)
	2014	Inhibits the PB2 cap-binding subunit of influenza A viruses RdRp	Phase 3 for influenza virus A	Clark et al.	(32)
Galidesivir	2014	Inhibits viral RdRp function by terminating non-obligate RNA chain	Phase 1 for yellow fever, Marburg virus, and COVID-19	Warren et al.	(15)
Baloxavir	2018	Inhibits cap-dependent endonuclease in PA unit of influenza virus RdRp	Influenza viruses A and B	Noshi et al.	(34)
Remdesivir	2018	Compete with ATA and terminates the nucleotide incorporation	Coronaviruses	Agostini et al.	(44)

AT-527	2019	Guanosine nucleotide prodrug with potent, pan-genotypic activity	HCV, Phase 2 for COVID-19	Berliba et al.	(45)
Emtricitabine & tenofovir alafenamide	2020	Inhibitor of reverse transcriptase and DNA chain elongation termination	COVID-19	Elfiky et al.	(20)
Emtricitabine & Tenofovir Disoproxil	2020	Iinhibitor of reverse transcriptase, and subsequently, it leads to DNA chain elongation termination	COVID-19	Copertino et al.	(25)
Clevudine	2020	Inhibit the HBV reverse transcriptase protein priming and DNA synthesis	The drug is being evaluated in phase 2 as a treatment for COVID-19 in Korea	Al-Horani & Kar	(46)
EIDD-2801	2020	The oxime form which mimics uridine and pairs with adenosine, while the other tautomer mimics cytosine and pairs with guanosine	Influenza, Ebola, Venezuelan equine encephalitis virus, MERS-CoV, SARS-CoV, SARS-CoV-2, and related zoonotic group 2b or 2c bat coronaviruses	Sheahan et al.	(27)

3.1.2 Remdesivir (or Veklury, GS-5734)

Remdesivir is a prodrug of a nucleoside analog that has antivirals with broad-spectrum properties [11]. Remdesivir provides two hydroxyl groups, strategies *that may be* employed as orally active through chemical modification and protectors by masking one of them as an ester, like modifying another.

Remdesivir has recently been promising to inhibit coronaviruses, including SARS-CoV and MERS-CoV, grown in *tissue culture* and animal models [12]. Compared to the Ebola virus RdRp, Remdesivir delays chain termination which is considered a possible mechanism for treating SARS-CoV and MERS-CoV [13]. Coronaviruses usually can correct mistakes, incorporating incorrect nucleoside analogs but Remdesivir has shown positive results to maintain its antiviral activity [14].

3.1.3 Galidesivir (Immucillin-A, BCX4430)

Galidesivir is an antiviral drug, an adenosine nucleoside analog that is like many nucleotide *analog* prodrugs inhibitor of RdRp activity. Galidesivir, such as prodrugs, is typically metabolized by cellular kinases to give the active form of the nucleoside triphosphate. The nucleoside *triphosphates* form binds to viral polymerases' active site and gets incorporated into the growing RNA strands, consequently disrupt chain termination [15, 16].

3.1.4 Ribavirin (Virazole)

Ribavirin is a nucleoside analogs containing guanosine [17]. The drug characteristics reflect *great* efforts against both viral RNA and DNA. For the antiviral activities, phosphorylation is activated to generate the triphosphate

nucleotide in a later phase that targets RNA synthesis and viral mRNA capping machinery [18]. The other mechanism of action is that Ribavirin-monophosphate induces depletion which occurs at guanosine triphosphate (GTP) level. A decreased intracellular GTP pool in host cells leads to decreased viral protein *production* and *reduces* viral genome replication [19]. Recently, computational modeling has demonstrated that ribavirin has a high binding affinity to RdRP of SARS-CoV-2 [20].

3.1.5 Clevudine (also known as Levovir and Revovir)

Clevudine is an antiviral drug which includes a range of thymidine nucleoside analog approved in Korea to treat hepatitis B virus infection [21]. Like previous agents, this prodrug requires intracellular phosphorylation modification to be in its active form, the triphosphate. Automatically, the active triphosphate forms appear to noncompetitive inhibition of the HBV reverse transcriptase protein and DNA replication [22].

3.1.6 Emtricitabine

Emtricitabine is a cytosine nucleoside analog which combines with Tenofovir Disoproxil or Tenofovir Alafenamide as a competitive inhibitor of human immunodeficiency virus-1 (HIV-1) and reverse transcriptase. It is activated via a kinase action-mediated phosphorylation to generate triphosphate form. Emtricitabine triphosphate is the active form that inhibits the HIV replication termination of chain elongation, and thus, it prevents the generation of complementary DNA (cDNA) [23]. In particular, tenofovir disoproxil (Viread; 2001) is known as an adenine-based acyclic nucleotide analog that reverses transcriptase inhibitors, and subsequently, it leads to termination of DNA chain elongation. The corresponding activation of the drug with the hydrolysis of the external esters spontaneously releases carbon dioxide and formaldehyde to form the tenofovir, subsequently it is submited to two phosphorylation steps to form diphosphate, the active drug [24]. Preventive efficacy of emtricitabine and tenofovir disoproxil as a prophylactic combination against infection SARS-CoV-2 is being evaluated in a randomized Clinical Trial (NCT04334928). The two drugs have been recognized as computational approaches and potential inhibitors of RdRP of SARS-CoV-2 [20, 25].

Likewise, tenofovir alafenamide is a nucleotide analog that, following activation, leads to competitive inhibition of RT and termination of DNA chain elongation. The drug activation process is different; however, several steps are similar to those of Remdesivir in cells infected by a series of bio-transformations [26]. Emtricitabine and tenofovir alafenamide is being tested as a prophylactic combination in COVID-19 patients being evaluated in randomized, double blind, placebo-controlled trial across the health care providers exposed to COVID-19 patients (NCT04405271, n=1378).

3.1.7 AT-527

AT-527 is a purine nucleotide prodrug that has showed potent antiviral activity against many

enveloped RNA viruses, including vector-borne RNA viruses that include dengue, human flaviviruses, and coronaviruses. After single-dose oral administration as Hemi-sulfate salt via metabolic activation, it can be converted to the monophosphate form.

3.1.8 EIDD-2801

EIDD-2801 is a prodrug form of the of β-D-N^4-hydroxycytidine that becomes hydrolyzed, releases the parental compound (EIDD-1931) over certain tissues with faster tri-phosphorylation, and coverts to the active triphosphate form to act against RdRP. The active form is integrated into the structures of RNA viruses; however, they occur in error catastrophe due to excessive mutations [27].

3.1.9 Sofosbuvir

Sofosbuvir was approved as an direct-acting antiviral (DAA) used in Hepatitis C (HCV) RdRP [28]. Some in silico and in vitro studies have shown that sofosbuvir and daclatasvir may also bind to the SARS-CoV-2 RdRP enzyme with a strong binding affinity [20, 29]. The drug is evaluated in a randomized, double-blinded controlled trial study to define its safety and efficacy in patients with mild symptoms of COVID-19.

3.1.10 Dasabuvir (ABT333), Beclabuvir and Pimodivir

Dasabuvir is a non-nucleoside inhibitor of NS5B viral RNA-dependent RNA polymerase in HCV [30]. Beclabuvir, a non-nucleoside inhibitor of the HCV RdRP, can most efficiently bind to RdRP SARS-CoV-2 and inhibit it [31]. Pimodivir (previously known as JNJ-63623872 or VX-787) is under progress for hospitalized patients with influenza A. Pimodivir is a non-nucleoside inhibitor subunitted for (PB2) influenza A virus polymerase complex resulting in significantly lower RNA synthesis.

Phase 3 Study of Pimodivir resulted in significant virologic response [32]. The computer-based study suggests that Dasabuvir, Pimodivir, and Beclabuvir may bind to SARS-CoV-2 RdRP with high affinity [20].

3.1.11 Baloxavir marboxil

Baloxavir marboxil belongs to the influenza antiviral drugs, developed in October 2018 to treat acute influenza; baloxavir CEN (cap-dependent endonuclease) can selectively inhibit the RNA replication [33]. A recent study of baloxavir revealed that the active form (BXA) hinders the viral RNA synthesis by using an enzymatic activity assay of CEN selectively that inhibits virus replication in infected cells with no cytotoxicity [34]. Regardless of the inhibitory effects of baloxavir acid on influenza treatment, so far there have been only some associated clinical trials on SARS – CO2 [8, 35].

3.2 RdRp domain

The following databases were searched based on our keyword groups related to RdRp. We systematically analyzed all articles

and discussed them in detail concerning the domain and subdomain of RdRp of RNA viruses. In fact, 54 articles were obtained from the literature search using
the search strategy described in the material and method section.

The International Committee on Taxonomy of Viruses classifies RNA viruses as double-stranded RNA (dsRNA) or single-stranded (ssRNA). In turn, the ssRNA viruses can be classified into viruses that have a positive sense (+RNA) and negative sense (-RNA), depending on the translation of primary genetic material. All RNA viruses employ dedicated replication and various strategies to amplify their genome [36]. One of the standard and unique strategies is a conserved RdRP domain. RdRP is one of the great targets of the drug for SARS-CoV-2, along with surface spike protein [37], which involves infection of human cells and its main protease viral polyproteins. The active catalytic site of the nsp12 RdRp consists of seven conserved motifs (A to G; Ftaigs. 1A and 3E and S6). Motifs A, B, C, and D, are from the palm subdomain, with an SDD sequence (residues 759 to 761) in motif C forming the active catalytic center. Both D760 and D761 coordinate the two magnesium ions at the catalytic center[38]. Motifs F and G are located within the finger subdomain; they interact with the template strand RNA and direct it into the active site. Motif F also interacts with the primer strand RNA, with the side chains of K545 and R555 contacting the +1 base and stabilizing the incoming nucleotide in the correct catalysis position. The protein structure of SARS-CoV-2 nsp12, the key component of the RdRp, combined with the cofactors nsp7 and nsp8, was recently determined by cryo-Electron Microscopy (cryo-EM) at 2.9-Å resolution [39]. In a comparative analytical model, it was demonstrated that the active triphosphate form of the nucleotide analog Remdesivir incorporates into the active site of nsp12 to inhibit the synthesis of the growing RNA strand and to terminate RNA synthesis [39]. Among 23 trials initiated from the systematic review, nine clinical trials are registered under the clinical trials registry for SARS-CO-2 therapeutics options. Five studies on lopinavir, ritonavir and arbidol, mesenchymal stem cells, hydroxychloroquine, traditional Chinese medicine and glucocorticoid therapy usage have instigated public participation. The four long-term studies incorporate analysis of antivirals, interferon atomization, cobicistat and darunavir, arbidol, and Remdesivir treatment for SARS-CO-2 patients. Junxiong Pang et al. explored the FDA accepted record of 7922 molecules and screened against the core polymerase with cofactors. They report a panel of FDA-approved drugs that demonstrate significant interactions with the active site's main amino acid residues. Remarkably, some of the recognized drugs (Lypressin, Ornipressin, Examorelin, Polymyxin B1) bind strongly with pockets of both forms of RdRP. Also, they also found robust candidates for the complex form which comprise Cistinexine, Cisatracurium, Nacortocin. These drugs have the probability to be measured while manufacturing therapeutic options [40]. Genome sequences of SARS-CoV-2 signify high similarity with other SARS corona viruses. Directing the RdRP active sites by antiviral drugs could be a potential therapeutic option for inhibition of coronavirus RdRp. Drug-based computer-generated and molecular docking results display that the antiviral Galidesivir and its

Table 2: Domain and subdomain of RdRp of RNA viruses.

Virus	Year	Authors	Abstract	Ref
COVID-19	2020	Yan Gao et al.	Nsp12, a member of the viral polymerase family, has a newly discovered -hairpin domain at its N terminus.	(47)
West Nile	2006	Hélène Malet et al.	The RdRp structure of Nile Virus was found to be most similar to the RdRp structures of the members of the Flaviviridae family, bovine viral diarrhea virus and hepatitis C virus.	(48)
Dengue	2007	Thai Leong Yap et al	The structure of the dengue virus reveals the presence of two motifs that are responsible for binding zinc ions. A chain-terminating nucleoside analog binds to the priming loop site in the absence of a template strand.	(49)
Bovine Viral Diarrhea	2006	Kyung H.Choi et al	The "N-terminal domain" of the similar polypeptide chain is shown to interact with the polymerase component by inspecting the crystal structure of BVDV RdRp, which includes a duplication of the amino acid Asn438.	(50)
Swine Fever	2006	Ming Xiao et al	The N-terminal domain of Swine Fever Virus NS5B plays an important role in template recognition and de novo RNA synthesis, as described by its crystal structure, which can be found in Swine Fever Virus.	(51)
Zika	2017	Andre S. Godoy et al	The recombinant ZIKV NS5 RdRp domain structure has a crystal structure that is very similar to the crystal structures of other flaviviral homologs.	(52)
Human Rhinovirus	2004	Robert ALove et al	The three crystal structures of viral serotypes (1B, 14, and 16) of HRV 3Dpol are quite similar to the structure of the 3Dpol enzyme found in closely related poliovirus (PV).	(53)
Japanese Encephalitis	2013	Guoliang Lu et al	The flavivirus NS5 crystal structure, which was obtained from the Japanese encephalitis virus, not only describes the distinct intra-molecular interactions that occur between MTase and RdRP, but it also completes the vision for polymerase motifs F and G.	(54)
Hepatitis C	1999	HideoAgo et al	Through the replication of positive ssRNA by viral RdRps, it is possible that a common structural discriminator of the template–primer duplex, which differentiates between DNA and RNA, might be characterized by distinct fingers. This finding was made possible by the crystal structure of the HCV RdRp.	(55)

anatomically similar compounds have shown potential effect against SARS-CoV-2 [41]. Using some computer-based methods, RdRP was targeted by Milbemycins (MMs), Ivermectin (IMT), Tadalafil (TF) and Baloxavir Marboxil (BM) a phosphodiesterase type 5 inhibitor. It is noteworthy that MM-A3 5-oxime (MMA35O), MM-A3 (MMA3), MM-A4 5-oxime (MMA45O), IMT, BM, and TF showed the highest potential binding affinity to RdRP (**Table 2**) [42].

4. Conclusion

The COVID-19 pandemic is a critical global health threat and no promising drug has yet been introduced for routine clinical use. Nevertheless, *research on different medications* has motivated enzymes that are potentially antiviral mediators for the natural viral course because they may diverge from host proteins. Among viral enzymes, RdRP is the main target of many present nucleotide drugs. In this paper, we reported the structure comparison and sequence alignment and recommended that the mode of substrate RdRp is extremely conserved in diverse RNA viruses. This provides a foundation for designing broad-spectrum antiviral drugs based on nucleotide analogs.

Acknowledgments

This study was financially supported by the Ardabil University of Medical Sciences. The authors would like to acknowledge all health-care workers who collaborated in the diagnosis and treatment of patients in corona wards in Iran.

Conflict of interest

The authors declare to have no conflict of interest.

Funding

This research was funded by IR.ARUMS.REC.1399.352.

References

[1] Adedeji AO, Marchand B, te Velthuis AJ, Snijder EJ, Weiss S, et al. Mechanism of nucleic acid unwinding by SARS-CoV helicase. *PloS one.* (2012) 7 (5).

[2] Ahn D-G, Choi J-K, Taylor DR,Oh J-W. Biochemical characterization of a recombinant SARS coronavirus nsp12 RNA-dependent RNA polymerase capable of copying viral RNA templates. *Archives of virology.* (2012) 157 (11): 2095-104.

[3] Adedeji AO, Marchand B, Te Velthuis AJ, Snijder EJ, Weiss S, et al. Mechanism of nucleic acid unwinding by SARS-CoV helicase. *PloS one.* (2012) 7 (5): e36521.

[4] Bressanelli S, Tomei L, Roussel A, Incitti I, Vitale RL, et al. Crystal structure of the RNA-dependent RNA polymerase of hepatitis C virus. *Proceedings of the National Academy of Sciences.* (1999) 96 (23): 13034-9.

[5] Agostini ML, Andres EL, Sims AC, Graham RL, Sheahan TP, et al. Coronavirus susceptibility to the antiviral remdesivir (GS-5734) is mediated by the viral polymerase and the proofreading exoribonuclease. *MBio.* (2018) 9 (2).

[6] Venkataraman S, Prasad BV,Selvarajan R. RNA dependent RNA polymerases: insights from structure, function and evolution. *Viruses.* (2018) 10 (2): 76.

[7] Campagnola G, Gong P,Peersen OB. High-throughput screening identification of poliovirus RNA-dependent RNA polymerase inhibitors. *Antiviral research.* (2011) 91 (3): 241-51.

[8] Li G,De Clercq E. Therapeutic options for the 2019 novel coronavirus (2019-nCoV). In: *Nature Publishing Group*. (2020).

[9] Furuta Y, Komeno T,Nakamura T. Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase. *Proceedings of the Japan Academy, Series B.* (2017) 93 (7): 449-63.

[10] Kiso M, Takahashi K, Sakai-Tagawa Y, Shinya K, Sakabe S, et al. T-705 (favipiravir) activity against lethal H5N1 influenza A viruses. *Proceedings of the National Academy of Sciences.* (2010) 107 (2): 882-7.

[11] Tchesnokov EP, Feng JY, Porter DP,Götte M. Mechanism of inhibition of Ebola virus RNA-dependent RNA polymerase by remdesivir. *Viruses.* (2019) 11 (4): 326.

[12] Sheahan TP, Sims AC, Graham RL, Menachery VD, Gralinski LE, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. *Science translational medicine.* (2017) 9 (396).

[13] Gordon CJ, Tchesnokov EP, Woolner E, Perry JK, Feng JY, et al. Remdesivir is a direct-acting antiviral that inhibits RNA-dependent RNA polymerase from severe acute respiratory syndrome coronavirus 2 with high potency. *Journal of Biological Chemistry.* (2020) 295 (20): 6785-97.

[14] Amirian ES,Levy JK. Current knowledge about the antivirals remdesivir (GS-5734) and GS-441524 as therapeutic options for coronaviruses. *One Health.* (2020): 100128.

[15] Warren TK, Wells J, Panchal RG, Stuthman KS, Garza NL, et al. Protection against filovirus diseases by a novel broad-spectrum nucleoside analogue BCX4430. *Nature.* (2014) 508 (7496): 402-5.

[16] Westover JB, Mathis A, Taylor R, Wandersee L, Bailey KW, et al. Galidesivir limits Rift Valley fever

virus infection and disease in Syrian golden hamsters. *Antiviral research.* (2018) 156: 38-45.

[17] Trang TP, Whalen M, Hilts- Horeczko A, Doernberg SB,Liu C. Comparative effectiveness of aerosolized versus oral ribavirin for the treatment of respiratory syncytial virus infections: a single- center retrospective cohort study and review of the literature. *Transplant Infectious Disease.* (2018) 20 (2): e12844.

[18] Cameron CE,Castro C. The mechanism of action of ribavirin: lethal mutagenesis of RNA virus genomes mediated by the viral RNA-dependent RNA polymerase. *Current opinion in infectious diseases.* (2001) 14 (6): 757-64.

[19] Te HS, Randall G,Jensen DM. Mechanism of action of ribavirin in the treatment of chronic hepatitis C. *Gastroenterology & hepatology.* (2007) 3 (3): 218.

[20] Elfiky AA. SARS-CoV-2 RNA dependent RNA polymerase (RdRp) targeting: An in silico perspective. *Journal of Biomolecular Structure and Dynamics.* (2020): 1-9.

[21] Jang JH, Kim JW, Jeong SH, Myung HJ, Kim H, et al. Clevudine for chronic hepatitis B: antiviral response, predictors of response, and development of myopathy. *Journal of viral hepatitis.* (2011) 18 (2): 84-90.

[22] Jones SA, Murakami E, Delaney W, Furman P,Hu J. Noncompetitive inhibition of hepatitis B virus reverse transcriptase protein priming and DNA synthesis by the nucleoside analog clevudine. *Antimicrobial agents and chemotherapy.* (2013) 57 (9): 4181-9.

[23] Frampton JE,Perry CM. Emtricitabine. *Drugs.* (2005) 65 (10): 1427-48.

[24] Murphy RA,Valentovic MA. Factors contributing to the antiviral effectiveness of tenofovir. *Journal of Pharmacology and Experimental Therapeutics.* (2017) 363 (2): 156-63.

[25] Copertino Jr DC, Lima B, Duarte R, Wilkin T, Gulick R, et al. Antiretroviral Drug Activity and Potential for Pre-Exposure Prophylaxis Against COVID-19 and HIV Infection. (2020).

[26] Birkus G, Bam RA, Willkom M, Frey CR, Tsai L, et al. Intracellular activation of tenofovir alafenamide and the effect of viral and host protease inhibitors. *Antimicrobial agents and chemotherapy.* (2016) 60 (1): 316-22.

[27] Sheahan TP, Sims AC, Zhou S, Graham RL, Pruijssers AJ, et al. An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. *Science translational medicine.* (2020) 12 (541).

[28] Merat S, Poustchi H, Hemming K, Jafari E, RADMARD AR, et al. polypill for prevention of cardiovascular disease in an urban iranian population with special focus on nonalcoholic steatohepatitis: a pragmatic randomized controlled trial within a cohort (polyiran-liver)–study protocol (study protocol). (2015).

[29] Beck BR, Shin B, Choi Y, Park S,Kang K. Predicting commercially available antiviral drugs that may act on the novel coronavirus (SARS-CoV-2) through a drug-target interaction deep learning model. *Computational and structural biotechnology journal.* (2020).

[30] Mantry PS,Pathak L. Dasabuvir (ABT333) for the treatment of chronic HCV genotype I: a new face of cure, an expert review. *Expert review of anti-infective therapy.* (2016) 14 (2): 157-65.

[31] Dutta K, Shityakov S, Morozova O, Khalifa I, Zhang J, et al. Beclabuvir can inhibit the RNA-dependent RNA polymerase of newly emerged novel coronavirus (SARS-CoV-2). (2020).

[32] Clark MP, Ledeboer MW, Davies I, Byrn RA, Jones SM, et al. Discovery of a novel, first-in-class, orally bioavailable azaindole inhibitor (VX-787) of influenza PB2. *Journal of medicinal chemistry.* (2014) 57 (15): 6668-78.

[33] Yoshino R, Yasuo N,Sekijima M. Molecular dynamics simulation reveals the mechanism by which the influenza cap-dependent endonuclease acquires resistance against baloxavir marboxil. *Scientific reports.* (2019) 9 (1): 1-8.

[34] Noshi T, Kitano M, Taniguchi K, Yamamoto A, Omoto S, et al. In vitro characterization of baloxavir acid, a first-in-class cap-dependent endonuclease inhibitor of the influenza virus polymerase PA subunit. *Antiviral research.* (2018) 160: 109-17.

[35] Lou Y, Liu L,Qiu Y. Clinical Outcomes and Plasma Concentrations of Baloxavir Marboxil and Favipiravir in COVID-19 Patients: an Exploratory Randomized, Controlled Trial. *medRxiv*. (2020).

[36] Ahlquist P. Parallels among positive-strand RNA viruses, reverse-transcribing viruses and double-stranded RNA viruses. *Nature Reviews Microbiology*. (2006) 4 (5): 371-82.

[37] Wu C, Liu Y, Yang Y, Zhang P, Zhong W, et al. Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. *Acta Pharmaceutica Sinica B.* (2020).

[38] Naqvi AAT, Fatima K, Mohammad T, Fatima U, Singh IK, et al. Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: Structural genomics approach. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease.* (2020): 165878.

[39] Gao Y, Yan L, Huang Y, Liu F, Zhao Y, et al. Structure of the RNA-dependent RNA polymerase from COVID-19 virus. *Science*. (2020) 368 (6492): 779-82.

[40] Ahmad J, Ikram S, Ahmad F, Rehman IU,Mushtaq M. SARS-CoV-2 RNA Dependent RNA polymerase (RdRp)–A drug repurposing study. *Heliyon.* (2020) 6 (7): e04502.

[41] Aftab SO, Ghouri MZ, Masood MU, Haider Z, Khan Z, et al. Analysis of SARS-CoV-2 RNA-dependent RNA polymerase as a potential therapeutic drug target using a computational approach. *Journal of translational medicine.* (2020) 18 (1): 1-15.

[42] Janabi AHD. Effective Anti-SARS-CoV-2 RNA Dependent RNA Polymerase Drugs Based on Docking Methods: The Case of Milbemycin, Ivermectin, and Baloxavir Marboxil. *Avicenna Journal of Medical Biotechnology*. (2020) 12 (4): 246.